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Comparison of the Association With Eczema Herpeticum in the Two Predominant Genotypes of Herpes Simplex Virus Type 1

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Eczema herpeticum, sometimes called Kaposi's varicelliform eruption, is usually caused by a disseminated herpes simplex virus infection in a patient whose underlying skin disease is atopic dermatitis. Herpes simplex virus type 1 (HSV-1), a widespread infectious agent in human populations, is the etiologic agent of eczema herpeticum. Analyses of restriction fragment length polymorphism (RFLP) of HSV-1 strains isolated in Japan, using restriction endonucleases, revealed the presence of two predominant genotypes of F1 and F35. The number of HSV-1 strains of F1 genotype was over twice that of the F35 genotype, and the nucleotide change between F1 and F35 was estimated to be 1.5%. The question of whether the genomic difference between two predominant genotypes could influence clinical manifestations remained to be addressed. On the basis of RFLP, we determined genotypes of HSV-1 strains isolated from the patients in Japan, including those with eczema herpeticum. Two of four HSV-1 strains of F35 genotype were from patients with eczema herpeticum, whereas none of 12 HSV-1 strains of F1 genotype was from those with eczema herpeticum. Thus, the F35 genotype seemed to be associated more frequently with eczema herpeticum than the F1 genotype.

KEY WORDS: Kaposi's varicelliform eruption, atopic dermatitis, restriction fragment length polymorphism (RFLP), restriction endonuclease, genetic variability

INTRODUCTION

Kaposi's varicelliform eruption is the enonym given to a distinct cutaneous eruption caused by herpes simplex virus (HSV) types 1 (HSV-1) and 2 (HSV-2), vaccinia virus, or Coxsackie A16 virus superimposed on a preexisting dermatosis [Mooney et al., 1994]. The most common is HSV infection with disseminated skin involvement, usually in patients with atopic dermatitis, and is termed eczema herpeticum. A striking increase in incidence of eczema herpeticum has occurred since 1980 [Bork and Bräuninger, 1988]; however, characterization of HSV strains isolated from patients with eczema herpeticum has not yet been done.

HSV-1 is an infectious agent widespread among human populations. Restriction endonuclease (RE) analysis of HSV genome DNA can clearly distinguish HSV-1 from HSV-2, and individual HSV-1 isolates can be identified [Buchman et al., 1978; Chaney et al., 1983]. Variations in RE cleavage patterns between HSV-1 strains, termed restriction fragment length polymorphism (RFLP), are due mostly to gain or loss of an RE cleavage site. The RFLP is used as a physical marker of the HSV-1 genome, and comparison of RFLP among HSV-1 strains facilitates determination of the epidemiology of HSV-1 infection [Sakaoka et al., 1984, 1986, 1995].

HSV-1 strains from geographically separate countries or anthropologically different races showed distinct patterns of RFLP, probably due to the nonepidemic nature of HSV-1 infection [Al-Ahdal et al., 1992; Rojas et al., 1993; Sakaoka et al., 1987, 1994; Umene et al., 1984; Umene, 1987]. HSV-1 tends to be latent, and transmission seems to be limited mostly to a relatively restricted geographic area. Therefore, HSV-1 strains with one genotype are assumed to have perpetuated and accumulated in a country or an area, probably forming a predominant genotype [Umene and Sakaoka, 1991]. Two predominant genotypes in Japan (F1 and F35) and one in Kenya have been identified [Sakaoka et al., 1987; Umene and Yoshida, 1993, 1994]. HSV-1 strains of a predominant genotype are assumed to have derived from a common ancestor of the predominant genotype; there-

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fore, the genomic characteristics of such strains should be similar. Strains belonging to one predominant genotype might possibly share biological properties and clinical manifestations common to the genotype. Differences in genomic characteristics between two predominant genotypes can cause some differences in biological properties and clinical manifestations.

We analyzed previously the RFLP of 66 HSV-1 strains isolated in Japan (Yoshida's collection) and found that 1 of 15 strains of F1 genotype and 2 of 7 strains of F35 genotype were from patients with eczema herpeticum [Umene and Yoshida, 1993]. In the present study, another set of 48 HSV-1 strains isolated in Japan (Sakaoka's collection) was analyzed to examine whether the F35 genotype is associated more commonly with eczema herpeticum than the F1 genotype. We found that none of the 12 strains of the F1 genotype and 2 of 4 strains of the F35 genotype were from patients with eczema herpeticum.

MATERIALS AND METHODS

Viruses

Forty-eight epidemiologically unrelated HSV-1 clinical strains (Sakaoka's collection) analyzed in the present study were isolated at a hospital in Kyoto, Japan. Working stocks of HSV-1 were made on Vero cells in Eagle's minimal essential medium with 2% fetal bovine serum at a low multiplicity of infection (0.01 PFU/cells). HSV-1 DNAs were prepared from Vero cells infected with HSV-1 as described previously [Umene et al., 1984; Sakaoka et al., 1987].

Restriction Endonuclease Digestion and Gel Electrophoresis

REs were purchased from Takara Shuzo Co. (Kyoto, Japan), and the conditions of digestions were as recommended by the manufacturer. The DNA digested with REs was separated in a 0.8% agarose gel, as described previously [Umene et al., 1984; Sakaoka et al., 1987]. The nomenclature of *Bam*HI, *Kpn*I, and *Sal*I fragments of HSV-1 was that of Locker and Frenkel [1979], as described elsewhere [Umene and Yoshida, 1993].

RFLP Markers for Classification of Genotype

Fifteen RFLP markers were used for the classification of genotype of HSV-1 strains (Table I). These RFLP markers have been defined elsewhere [Umene and Yoshida, 1993] and were adequate for selecting HSV-1 strains belonging to each predominant genotype of F1 and F35 [Sakaoka et al., 1987; Umene and Yoshida, 1993, 1994].

RESULTS

We analyzed earlier the RFLP of 66 HSV-1 strains isolated at hospitals in Tokyo and Osaka, Japan (Yoshida's collection), using three REs of *Bam*HI, *Kpn*I, and *Sal*I [Umene and Yoshida, 1993]. Fifteen strains were classified into the most predominant genotype of F1, and 7 strains belonged to the second most predominant genotype of F35. One of fifteen HSV-1 strains of F1 genotype (7%) and 2 of 7 HSV-1 strains of F35 genotype (29%) were from patients with eczema herpeticum (Table II).

In the present study, we analyzed another set of 48 HSV-1 strains isolated at a hospital in Kyoto, Japan (Sakaoka's collection), the objective being to test the hypothesis that HSV-1 strains of F35 genotype are associated more frequently with eczema herpeticum than those of the F1 genotype. We digested DNAs of the 48 HSV-1 strains with *Bam*HI, *Kpn*I, and *Sal*I; electrophoresed the digested DNAs in agarose gels; and identified RFLP. HSV-1 strains belonging to each predominant genotype of F1 and F35 were searched for on the basis of distribution of the 15 RFLP markers (Table I). This approach proved adequate for the determination of F1 and F35 genotypes [Sakaoka et al., 1987; Umene and Yoshida, 1993, 1994]. Twelve HSV-1 strains were classified into the F1 genotype, and none (0%) was from patients with eczema herpeticum (Table II). Four HSV-1 strains were classified into the F35 genotype, and two (50%) were from patients with eczema herpeticum (Table II). Thus, strains of F35 were isolated more frequently from patients with eczema herpeticum than those of F1.

On the supposition (null hypothesis denoted by H_0) that the ratio of F1 strains from patients with eczema herpeticum to the total strains of F1 genotype is the same as that of the F35 genotype, the probability of results observed concerning the F1 genotype was calculated [Spiegel, 1961]. In our previous study analyzing Yoshida's collection [Umene and Yoshida, 1993], 1 of 15 F1 strains and 2 of 7 F35 strains were from patients with eczema herpeticum (Table II). If the ratio of F1 strains from eczema herpeticum to total strains was 2:7, as was observed for the F35 genotype, then the probability that one (P_1) or fewer [i.e., zero (P_0)] F1 strain is from patients with eczema herpeticum is $P_0 + P_1 = {}_{15}C_0(2/7)^0(5/7)^{15} + {}_{15}C_1(2/7)^1(5/7)^{14} = 0.045$ (<0.05). Because the probability is 0.045, which is less than 0.05, we do not accept the supposition H_0.

In the set of 48 HSV-1 strains analyzed in the present study (Sakaoka's collection), none of 12 F1 strains and 2 of 4 F35 strains were from patients with eczema herpeticum (Table II). If the ratio of F1 strains from eczema herpeticum to total strains was 2:4, as was observed for the F35 genotype, then the probability that no (P_0) F1 strain is from patients with eczema herpeticum is $P_0 = {}_{12}C_0(1/2)^0(1/2)^{12} = 0.00024$ (<0.05). Because the probability is 0.00024, which is much less than 0.05, we do not accept the supposition H_0.

When two sets of HSV-1 strains (Yoshida's collection and Sakaoka's collection) were combined, 1 of 27 F1 strains and 4 of 11 F35 strains were from patients with eczema herpeticum (Table II). If the ratio of F1 strains from eczema herpeticum to total strains was 4:11, as was observed for the F35 genotype, the probability that one (P_1) or fewer [i.e., zero (P_0)] F1 strains is from patients with eczema herpeticum is $P_0+P_1 = {}_{27}C_0(4/11)^0(7/11)^{27} + {}_{27}C_1(4/11)^1(7/11)^{26} = 0.000082$ (<0.05). Because the probability is 0.000082, a value much less than 0.05,

TABLE I. RFLP Markers Used to Genotype HSV-1 Strains

Variation[a] No.	Definition[b]	Genotype[c] F1	F35
VR11	Gain of the *Sal*I site between fragments I and C (and F)	−	+
VR25	Loss of the *Sal*I site between fragments Z and H′	−	+
VR23	Gain of a *Kpn*I site on fragment E generating two fragments of 5.5 and 5.7 kb	−	+
VR21	Loss of the *Bam*HI site between fragments A′ and A	−	−
VR22	Gain of a *Bam*HI site on fragment A generating two fragments of 1.7 and 9.5 kb	−	−
VR3	Loss of the *Kpn*I site between fragments Ma and Mb	−	+
VR6	Gain of the *Bam*HI site between fragments W and K′	−	+
VR7	Loss of the *Bam*HI site between fragments D and H	−	−
VR61	Smaller *Bam*HI-0 fragment of 3.7 kb instead of 3.9 kb	−	−
VR8	Loss of the *Sal*I site between fragments K and C′	−	+
VR64	Gain of a *Kpn*I site on fragment Aa generating two 5.0 kb fragments	−	−
VR9	Loss of the *Kpn*I site between fragments Aa and Ab	−	−
VR73	Gain of a *Sal*I site on fragment Q generating two fragments of 3.5 kb and 0.6 kb	−	+
VR10	Gain of the *Kpn*I site between fragments Ab and Y	−	+
VR72	Loss of the *Kpn*I site between fragments T and O	−	+

[a]Variations (RFLP markers) have been defined elsewhere [Umene and Yoshida, 1993].
[b]Nomenclature of restriction fragments is that of Locker and Frenkel [1979], as described previously [Umene and Yoshida, 1993].
[c]Genotypes F1 and F35 are defined on the basis of 15 RFLP markers listed here [Sakaoka et al., 1987; Umene and Yoshida, 1993, 1994].

TABLE II. Genotype and Number of HSV-1 Strains From Patients With Eczema Herpeticum

Genotype[a]	No. of HSV-1 strains (No. of HSV-1 strains from patients with eczema herpeticum) Yoshida's collection[b]	Sakaoka's collection[c]
F1	15 (1)	12 (0)
F35	7 (2)	4 (2)
others	44 (2)	32 (7)

[a]Genotype of HSV-1 strains was determined on the basis of RFLP markers listed in Table I.
[b]RFLP of 66 HSV-1 strains (Yoshida's collection) isolated in Tokyo and Osaka analyzed in a previous study [Umene and Yoshida, 1993].
[c]RFLP of 48 HSV-1 strains (Sakaoka's collection) isolated in Kyoto analyzed in the present study.

we do not accept the supposition H_0. Therefore, results of analyses of two HSV-1 collections were supportive of the hypothesis that F35 strains may be associated more frequently with eczema herpeticum than are F1 strains.

DISCUSSION

The genetic variability of viruses facilitates differentiation and classification of strains, and examination of virus evolution and mode of transmission and distribution is thus made feasible. The association of virus strains of a particular genotype with biological characteristics and clinical manifestations has been examined and evidence supporting such an association obtained; e.g., variants of Epstein-Barr virus may be associated with the development and/or maintenance of head and neck tumors [Jeng et al., 1994]; some differences in the distribution of human polyomavirus BK virus subtypes between clinical groups were noted [Jin et al., 1993]; some cases of chronic fatigue syndrome were possibly caused and perpetuated by a novel enterovirus [Galbraith et al., 1995]; and antibody responses toward specific hepatitis C virus (HCV) proteins and responses to interferon therapy were seen to depend on HCV genotypes [Yuki et al., 1995]. Therefore, the difference in genotype of herpes simplex virus (HSV) possibly influences biological characteristics and clinical manifestations.

Recurrence patterns of HSV infection differ according to viral antigenic type and anatomical site; i.e., oral-labial recurrences are more frequent with HSV-1 than with HSV-2, and genital recurrences are more frequent with HSV-2 than with HSV-1 [Lafferty et al., 1987]. Two predominant genotypes of F1 and F35 are present in HSV-1 strains isolated in Japan, and the nucleotide change between F1 and F35 was estimated to be 1.5% [Umene and Yoshida, 1993]. Such differnces in nucleotide sequences may influence biological characteristics and clinical manifestations. We analyzed two sets of HSV-1 strains isolated in Japan, and propose that HSV-1 strains of F35 genotype may be associated more frequently with eczema herpeticum than those of the F1 genotype (Table II). Therefore, the results derived from the present study prepare the background for further studies concerning relations of genomic characteristics in strains with biological characteristics and clinical manifestations.

The majority of HSV-1 strains isolated in Japan were

classified into genotypes other than two predominant genotypes (Table II). The number of strains belonging to each genotype other than the predominant genotypes is smaller than that belonging to each predominant genotype [Sakaoka et al., 1987; Umene and Yoshida, 1993]. Therefore, statistical analysis of the number of strains of a genotype other than the predominant genotype would be more difficult than statistical analysis of the predominant genotype; hence, we dealt with strains of the predominant genotype in the present study. Two predominant genotypes appeared to differ in association with eczema herpeticum (Table II). Ongoing studies will address the problem of whether the genotype other than the predominant genotype might differ from the others, in association with the eczema herpeticum.

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